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CURRENT OPINION

'No cyst, no echinococcosis': a scoping review update on the diagnosis of cystic echinococcosis after the issue of the WHO-IWGE Expert Consensus and current perspectives

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Purpose of review
In 2010, the WHO-Informal Working Group on Echinococcosis (IWGE) published an Expert Consensus on the diagnosis and treatment of echinococcal infections. We provide an update on the diagnosis of cystic echinococcosis through a scoping review of the literature published after the release of the WHO-IWGE document.

Recent findings
Ultrasound accurately and reliably depicts the pathognomonic signs of cystic echinococcosis (CE) stages compared with other imaging techniques. Among these, T2-wighted MRI is to be preferred to computed tomography, which has poor performance for the etiological diagnosis of CE. A negative serology cannot exclude the diagnosis of CE, while a positive serology, applied after the visualization of a CE-compatible lesion, may confirm a CE diagnosis. Serology alone must not be used to define 'CE' nor as 'screening' tool for infection. Other imaging and laboratory techniques did not show clinically applicable performances.

Summary
In the absence of a focal lesion compatible with a CE cyst, no diagnosis of CE should be attempted. There is urgent need to achieve univocal CE case definitions and consensus diagnostic algorithm, as well as standardization of diagnostic methods and issue of a Target Product Profile of CE diagnostics, as advocated by the WHO in the 2021–2030 roadmap for neglected tropical diseases (NTDs).

Keywords
cystic echinococcosis, imaging, laboratory diagnosis, serology

INTRODUCTION

In the intermediate host, including humans, the larval stage (metacestode) of *Echinococcus granulosus sensu lato*, causing cystic echinococcosis (CE), develops as a well delimited cystic lesion (with the exception of the osseous localization, where infiltrative growth occurs [1]). CE cysts may present with variable morphology, classified in stages as schematized in Fig. 1 [2]. Notably, there is a fairly good correspondence between these stages and the biological viability for what concerns CE1-CE2-CE3b cysts (biologically viable) and CE4-CE5 cysts (inactive/not viable) [3]. Exceptions are CE3a cysts, which can be viable or not viable [3], and a proportion of CE4 cysts, which can be still viable in a variable percentage of cases [4–6]. Currently, no assay can identify what individual CE3a or CE4 cyst is actually viable or not viable, implying the need for years-long follow-up with imaging to visualize morphological changes reflecting viability outcomes. The correct etiological identification of a focal lesion is of paramount importance to implement the appropriate clinical management and avoid

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KEY POINTS

- In the absence of a focal lesion compatible with a cystic echinococcosis (CE) cyst, no diagnosis of cystic echinococcosis should be attempted.
- Ultrasonography accurately and reliably depicts the pathognomonic signs of CE cyst stages compared with other imaging techniques; among these, T2-wighted MRI is to be preferred to CT.
- A negative serology cannot exclude the diagnosis of cystic echinococcosis, since in many conditions, cystic echinococcosis is associated with seronegative results, while a positive serology, applied only after the visualization of a CE-compatible lesion, may confirm a diagnosis of cystic echinococcosis.
- Serology alone must not be used to define 'CE' nor as a 'screening' tool for infection, because the higher rate of seropositivity in population studies does not reflect high sensitivity but rather low specificity of serology when applied to an infection with low prevalence and therefore low pretest probability such as cystic echinococcosis.

misdiagnoses and mistreatment, which can have devastating consequences for the patient. However, despite the worldwide distribution of cystic echinococcosis, its diagnosis is still difficult, and a consensus diagnostic algorithm is unavailable. The main difficulties in the diagnosis of cystic echinococcosis are reaching a conclusive etiological diagnosis by noninvasive methods and evaluating CE cyst viability of CE3a and CE4 stages, which are pivotal for clinical decision-making.

In 2010, the WHO-Informal Working Group on Echinococcosis (IWGE) published an Expert Consensus on the diagnosis and treatment of echinococcal infections [2], which included the cyst classification and case definitions. This article aims to provide an update on the diagnosis of cystic echinococcosis by means of a scoping review of the literature published after the release of this document [2]. We focus on tools for the diagnosis of patients with uncomplicated cystic echinococcosis, which is both the most common and probably the most diagnostically problematic clinical presentation. Studies not including diagnostic tools applied on humans/human samples and methods applied to cyst material obtained invasively, will not be reviewed here. So far, seroassays have proven poorly reliable for defining clinical outcome during the follow-up, which remains based on imaging, and this aspect would not be specifically reviewed here either.

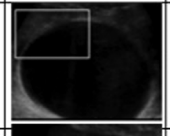
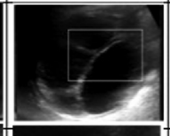
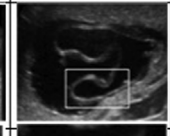
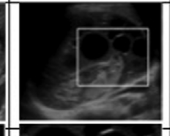
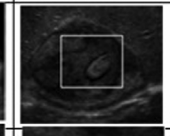
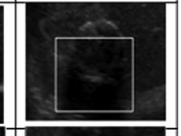
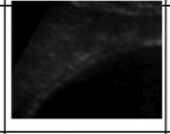
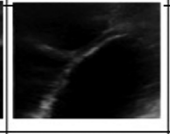
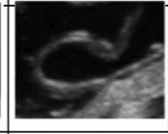
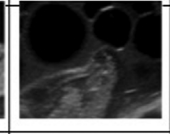
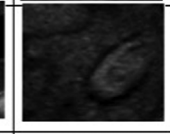
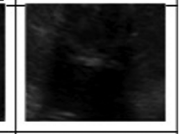
WHO-IWGE cyst stage	CE1	CE2	CE3a	CE3b	CE4	CE5
Ultrasound image of hepatic cyst						
Box: zoom of pathognomonic sign						
Viability	Viable	Viable	Viable/ not viable	Viable	Not viable/ low viability	Not viable
Activity group	Active	Active	Transitional (from active to inactive)	Transitional (reactivation of an inactive stage)	Inactive	Inactive
Description of pathognomonic signs	Double wall sign	Daughter cysts with adjacent walls	Detached parasitic layers	Daughter cysts in solid matrix with folded parasitic layers	Solid matrix with folded parasitic layers	Solid matrix with folded parasitic layers and evident egg-shell calcification

FIGURE 1. CE cyst stages according to the WHO-IWGE classification [2], viability, activity status, and description of pathognomonic signs on ultrasound.

LITERATURE SEARCH

On 6 February 2023, we performed a PubMed (MEDLINE) literature search using the strategy reported in Supplementary file 1, http://links.lww.com/COID/A45. We restricted the search to human studies published after April 2010, when the WHO-IWGE Expert Consensus [2] was published. No language restriction was applied.

Original prospective and retrospective cohorts, case–control, cross-sectional and diagnostic accuracy studies, as well as systematic reviews of the topics of interest were reviewed. When the full text was not available, data were extracted from the abstract, when possible. The flow diagram of the electronic searches and selection of publications is shown in Fig. 2. The full list of papers finally included in this review is available in Supplementary file 2, http://links.lww.com/COID/A46.

UPDATES ON IMAGING TECHNIQUES FOR THE ETIOLOGICAL DIAGNOSIS AND STAGING OF ECHINOCOCCAL CYSTS

In uncomplicated cystic echinococcosis, especially in the liver, diagnosis and cyst staging are two inseparable aspects, as cystic echinococcosis stages are characterized by pathognomonic features, the recognition of which allow both etiological

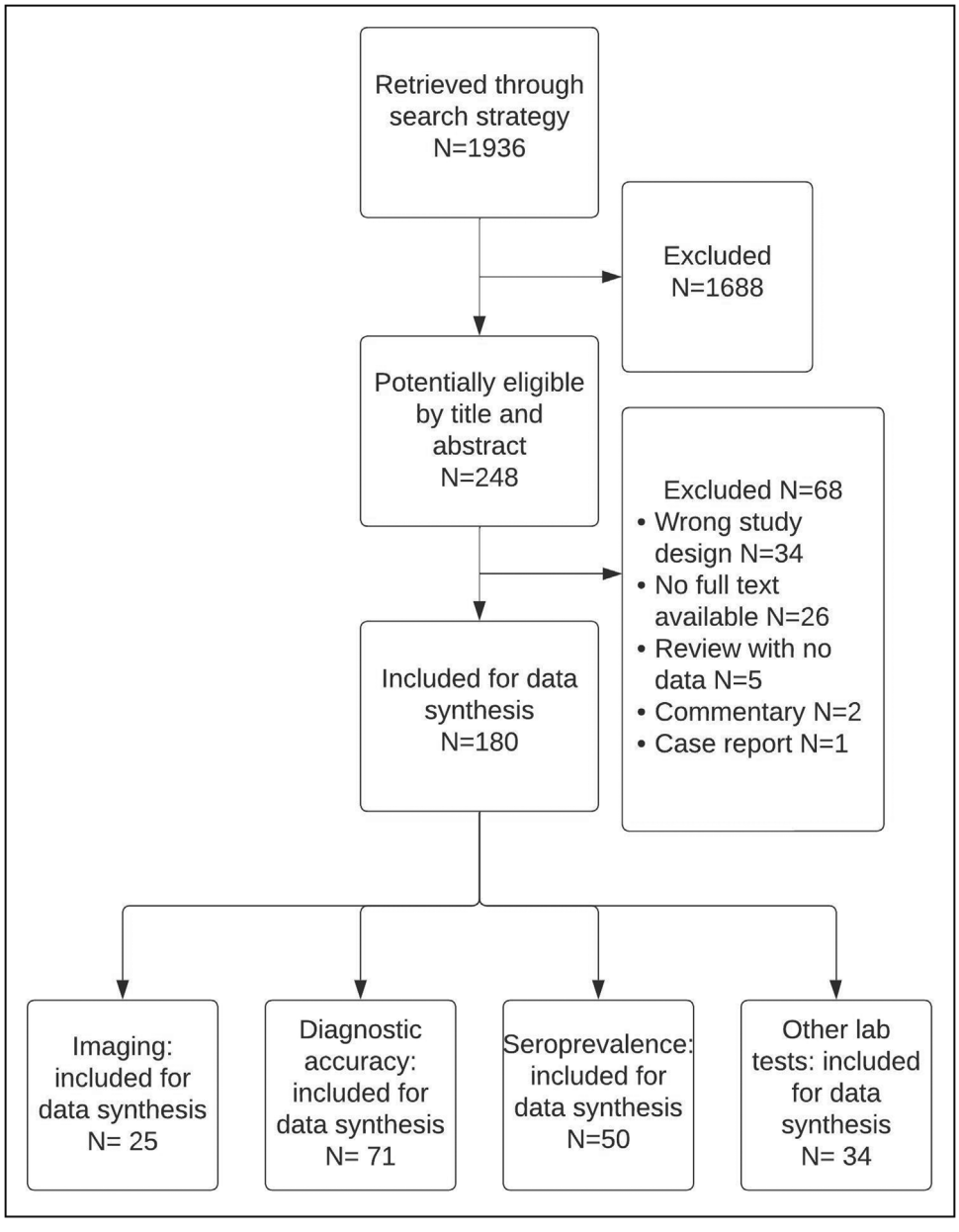


FIGURE 2. Literature search and selection.

diagnosis and staging (Fig. 1). On the basis of these features, differential diagnosis and clinical decision-making are based.

Ultrasonography, by which CE cyst stages are defined, accurately and reliably depicts the pathognomonic signs of CE cyst stages, both when compared with histopathology of surgically removed cysts [7], and to other imaging techniques [8▪▪]. The seminal study by Stojkovic *et al.* [8▪▪] rigorously evaluated the agreement on cyst staging between three imaging modalities, demonstrating that MRI had almost perfect agreement (Cohen's Kappa 0.8–1) with ultrasonography, while agreement of CT was only moderate (Cohen's Kappa 0.6–0.7). In particular, heavily T2-weighted sequences have excellent performance for cyst staging [8▪▪]. On the contrary, diffusion-weighted MRI (DW-MRI) performs poorly [9,10]. T2-weighted pulmonary MRI was also found having good performance compared with CT for the etiological diagnosis of lung cystic echinococcosis [11–13]. As known, CT better detects cyst calcifications [8▪▪,11,14]. However, the presence of peripheral 'egg-shell' calcification is not pathognomonic of CE cysts, therefore cannot, alone, lead to the diagnosis of cystic echinococcosis; on the contrary, the presence of isolated intra-lesion calcifications can exclude the diagnosis of cystic echinococcosis. Similarly, contrast enhancement of cyst components can exclude cystic echinococcosis, as the metacestode is not vascularized, but, clearly, absence of contrast-enhancement cannot confirm the diagnosis of cystic echinococcosis. The unreliable performance of CT in depicting signs diagnostic for cystic echinococcosis and in differentiating cyst stages, which need different management approaches, clearly make this technique less adequate than ultrasonography and MRI for the work-up of suspect cystic echinococcosis.

Differential diagnosis of CE cysts is wide, ranging from simple cysts to neoplasms. Of particular importance is the differential diagnosis with alveolar echinococcosis caused by *Echinococcus multilocularis*, especially in co-endemic areas, as cystic echinococcosis and alveolar echinococcosis are two very different diseases and dramatic consequences may derive from their inaccurate differentiation [15]. Several studies published in the last decade have explored the usefulness of advanced imaging techniques, including ultrasound elastography, CT, and DW-MRI, for the differential diagnosis of cystic echinococcosis and other lesions; however, results were variable and overall disappointing when coming to clinical usefulness [16–23]. FDG-PET/CT, which has a defined role in alveolar echinococcosis, has been also applied to cystic echinococcosis, with potential usefulness to evaluate the presence of inflammatory complications [24–26], but not for evaluation of CE cysts in terms of viability [27].

The interpretation (and execution, in the case of ultrasonography) of imaging techniques towards the recognition of pathognomonic signs of cystic echinococcosis (or which exclude cystic echinococcosis) is operator-dependent. Recently, several studies have tried to apply automatic classification algorithms to ultrasonography and CT images, to improve the diagnosis of cystic echinococcosis also by nonexpert personnel. When applied to the differential diagnosis of cystic echinococcosis vs. alveolar echinococcosis using CT, performance was clinically unsatisfactory [28▪▪]. Studies based on ultrasonography images aiming to automatically classify cyst stages had variable accuracy [29▪,30▪], but did not address the main interest of automatic imaging interpretation, that is differential diagnosis. On the contrary, the reliability of operators in recognizing CE cysts pathognomonic signs and stages by ultrasonography has been evaluated with positive results. When experts on ultrasonography diagnosis of cystic echinococcosis were involved in a study aiming to evaluate the reliability of WHO-IWGE US classification of CE cysts [31], it was found a substantial to almost perfect inter-observer and intra-observer agreement, confirming that experts can reliably identify and stage CE cysts based on ultrasonography. In the context of a four decades-long control programme for cystic echinococcosis in Argentina, using ultrasonography since 1997 as the only diagnostic tool for cystic echinococcosis population screening [32], a yearly FASE (Focused Assessment with Sonography for Echinococcosis) short course is used since year 2000 to train rural physicians [33▪▪]. The authors reported that on the first screening performed by trainees immediately after the course, all suspected cases, re-evaluated by an experienced operator, were not confirmed as cystic echinococcosis. This stresses that both technical ultrasonography skills and specific knowledge and experience in recognizing cystic echinococcosis pathognomonic imaging features are required for an accurate diagnosis. However, the authors also discuss that the cost of this counter-evaluation was lower than that which would be caused by complications of undiagnosed cystic echinococcosis.

UPDATES ON SEROLOGY AND OTHER LABORATORY TOOLS FOR THE DIAGNOSIS OF ECHINOCOCCAL CYSTS

In the WHO-IWGE Expert Consensus document [2], the application of 'high-sensitivity serological tests, confirmed by a separate high specificity serological test' is listed among diagnostic criteria for cystic

echinococcosis. More recent studies allowed better framing several aspects of the serodiagnosis of cystic echinococcosis, such as the variables associated with seroassays' results, the comparative performance of different seroassays and the rationale application of seroassays within the diagnostic work-up.

Seroassays for cystic echinococcosis are extremely heterogeneous in terms of format, antigen(s) used and robustness, as highlighted by recent studies comparing different assays or the same assay in different laboratories [34▪,35▪,36▪,37▪]. This stresses the recommendation that centres performing serology for cystic echinococcosis should evaluate the performance of tests they use, calculating accuracy and posttest probabilities in their own epidemiological context, by analysis of sera from clinically well characterized cohorts. Well known causes of cross-reactivity in seroassays for cystic echinococcosis include mainly *E. multilocularis* and *Taenia solium*/cysticercosis [38,39], among others. Furthermore, in endemic regions, it is well known that individuals seropositive to *E. granulosus* markedly outnumber actual cases of cystic echinococcosis. This is confirmed by recent population studies and hospital-based cohorts retrieved by this review [40–48], with seropositivity being found in a large proportion of imaging-negative people (two to eight times higher seroprevalence than prevalence of actual infection with the metacestode), including in areas where no cystic echinococcosis cases were detected. This seropositivity-only condition has been suggested to be in part due to 'exposure' to the parasite, but it appears not predictive of the future development of a CE cyst [49]. The parasite-specific antibody profiling of these seropositive imaging-negative individuals showed a profile compatible with a poorly specific immune response [50]. The misleading outcome of false positivity can be at least partially overcome by the application of serology only *after* a lesion compatible with cystic echinococcosis is visualized. This strategy increases the pretest probability of cystic echinococcosis infection and therefore improves the posttest probability result of the seroassay, especially if more than one test based on different antigens is applied [35▪,37▪,51]. False-negative results can be more difficult to cope with. False-negative results in confirmed cystic echinococcosis cases have been robustly associated with CE cyst stage (CE1 and CE4-CE5 stages vs. CE2-CE3a-CE3b), independently of the test format and antigen(s) used [52▪▪]. Other factors that are associated with higher proportion of false-negative serology results are CE cyst localization (extra-hepatic vs. hepatic) [53–65], size (small vs. large), number (single vs. multiple) and integrity (untreated/complicated vs. treated/complicated) [39,57,60,65,66]. In diagnostic accuracy and hospital cohort studies retrieved in this review [53–65], seropositivity rates in individuals with lung cystic echinococcosis ranged from 80% at best to as low as 17%, or 12% in individuals with cysts in uncommon localizations. These figures are always lower that those obtained with hepatic cystic echinococcosis. Despite their importance in seroassays' results, these variables are infrequently reported in published studies, hampering a precise evaluation of their performances. Of 71 diagnostic accuracy studies retrieved by this review and available for data extraction (Supplementary file 3, http://links.lww.com/COID/A47), only 23 (32.4%) described the samples cohort in terms of cyst stage, 40 (56.3%) described the localization of the cysts and 28 (40%) mentioned whether the serum was collected before or after treatment. Of note, only 22 out of 66 publications (33.8%) aiming to assess also test specificity included a clinically relevant control group (i.e. patients with other focal lesions, alveolar echinococcosis and so on). In synthesis, therefore, a negative serology cannot exclude the diagnosis of cystic echinococcosis while a positive serology, if carried out with validated assays and applied only after the visualization of a CE-compatible lesion, may confirm a diagnosis of cystic echinococcosis. Confirmation can also be achieved through observation of seroconversion and/or change in cyst morphology after treatment of a CE-compatible cyst with liquid content (*ex-juvantibus*).

Taken together, these results confirm and reinforce the indication that serology alone must not be used to define 'CE' nor as a 'screening' tool for infection, as the higher rate of seropositivity in population studies does not reflect high sensitivity but rather low specificity of serology for cystic echinococcosis, an infection with low-prevalence and therefore low pretest probability. Furthermore, its theoretical use for 'early diagnosis' or for 'capturing active cysts' is thwarted by its low sensitivity especially for 'young' CE1 cysts and by its positivity also in a proportion of cases with inactive CE cysts. Finally, its theoretical use for 'screening for CE in localizations not explorable by US' is contradicted by the low sensitivity for extra-hepatic cystic echinococcosis. Unfortunately, studies using only serology are still carried out and published [18/61 (29.5%) of population/surveillance-based surveys published in the target period of this review] (Supplementary file 3, http://links.lww.com/COID/A47), which provide uninformative data on prevalence of infection in a population.

Other diagnostic assays different than those detecting antibodies have been applied for the diagnosis of cystic echinococcosis, or the definition of

cyst's viability or potential usefulness for follow-up (Supplementary file 3, http://links.lww.com/COID/A47). Of the 34 studies published in the target timeframe of this review, $n=9$ investigated genomic/miRNA targets, $n=8$ serum host-derived markers, $n=6$ antigen detection, $n=5$ proteomics/metabolomics, $n=4$ in-vitro cytokine release and $n=2$ spectrometric analyses. With the caveat that we did not evaluate here the study design in terms of cystic echinococcosis and control cohort characteristics, in general, these markers seem still quite far from possible use in practice. Of the 32 studies from which data were available, nine (28.1%) did not report practically applicable cut-off/accuracy data, and of the 23 studies wherein such practical interpretation was provided, 15 (65.2%) reported low (<80%) sensitivity. The other eight (34.8%) studies reporting higher sensitivities [67–74] encompassed very different targets, with antigen detection and PCR as the diagnostic techniques most easily implementable, should any of these targets be further validated and reach clinical practice.

THE WAY FORWARD TOWARDS THE FORMALIZATION OF CONSENSUS CYSTIC ECHINOCOCCOSIS CASE DEFINITIONS AND DIAGNOSTIC ALGORITHM

The WHO-IWGE Expert Consensus [2] included clinical and diagnostic criteria on which possible, probable and confirmed cystic echinococcosis cases were defined. However, in the light of current data available, these criteria need revision. For example, pathognomonic ultrasonography features were not taken into account and only intervention-related analyses (microscopy or molecular biology on invasively collected material; response to treatment) were envisaged in the definition of 'confirmed CE'. Furthermore, 'possible CE' could be defined as seropositivity-only. In the opinion of who writes, a more practically useful definition of 'confirmed' vs. 'suspect' cystic echinococcosis is needed, which takes, in the first place, from the identification of an actual lesion compatible with cystic echinococcosis.

To complicate things further, other sources of data inaccuracy hamper the estimate of disease burden from official records. For example, in Europe, heterogeneous requirements apply for reporting cystic echinococcosis vs. alveolar echinococcosis as compared to 'echinococcosis' in general [75] and the cystic echinococcosis case definition included in current legislation is not even in line with that currently provided by the WHO, as it envisages only the 'confirmed case' category and this is also defined by seropositivity-only [76].

DISCUSSION/CONCLUSION

The diagnosis of cystic echinococcosis should be based on imaging (i.e. in the absence of a focal lesion compatible with a CE cyst, no diagnosis of cystic echinococcosis case should be attempted), while serology has a complementary role for cystic echinococcosis confirmation (but not for ruling cystic echinococcosis out). Clinical management of uncomplicated cystic echinococcosis depends on correct etiological/differential diagnosis and staging [2], and devastating consequences may derive from the misdiagnosis and mistreatment of cystic echinococcosis and other lesions entering in differential diagnosis [15]. There is therefore an urgent need to achieve univocal case definitions, to be then received by national and international stakeholders, and to achieve consensus on a diagnostic algorithm. The WHO-IWGE is carrying out, at the time of writing, a Delphi consensus study to this aim. Standardization of diagnostic methods is also terribly needed. Ultrasonography has been demonstrated to be reliable in expert hands [31], but is an operator-dependent exam and standardized cystic echinococcosis-focused training schemes would be advisable. Seroassays are not standardized, have variable performance and too often are not validated using an appropriate panel of 'local' sera. The actual issue of a Target Product Profile (TPP) of cystic echinococcosis diagnostics, as now clearly advocated by the WHO in the 2021–2030 roadmap on NTDs [77] will forcibly provide direction in this field, but so far TPP definition is not being worked on.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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